Mechanisms of Insulin Resistance in Aging

RAYMOND I. FINK, ORVILLE G. KOLTERMAN, JENNIFER GRIFFIN, and JERROLD M. OLEFSKY, *Department of Medicine, University of Colorado Health Sciences Center, Division of Endocrinology; Denver Veterans Administration Hospital, Denver, Colorado 80262*

ABSTRACT We have studied 17 elderly and 27 nonelderly, nonobese subjects (mean age 69±1 and 37±2 yr, respectively) to assess the mechanisms responsible for the abnormal carbohydrate tolerance associated with aging. Serum glucose and insulin levels were significantly elevated in the elderly subjects compared with the nonelderly subjects during a 75-g oral glucose tolerance test, suggesting an insulin resistant state. Peripheral insulin sensitivity was assessed in both groups using the euglycemic glucose clamp technique during an insulin infusion rate of 40 mU/m^2 per min. Similar steady-state serum insulin levels led to a peripheral glucose disposal rate of 151±17 mg/m^2 per min in the elderly compared with a value of 247±12 mg/m^2 per min in the nonelderly, thus documenting the presence of insulin resistance in the elderly subjects. Insulin binding to isolated adipocytes and monocytes was similar in the elderly and nonelderly groups (2.34±0.33 vs. 2.62±0.24% and 5.04±1.10 vs. 5.12±1.07%), respectively. Thus, insulin resistance in the presence of normal insulin binding suggests the presence of a postreceptor defect in insulin action. This was confirmed by performing additional euglycemic clamp studies using infusion rates of 15 and 1,200 mU/m^2 per min to assess the contours of the dose-response relationship. These studies revealed a 39 and 25% decrease in the glucose disposal rate in the elderly subjects, respectively. The results confirm the presence of a postreceptor defect as well as a rightward shift in the dose-response curve. Insulin's ability to suppress hepatic glucose output was less in the elderly subjects during the 15 mU/m^2 per min insulin infusion (77±5 vs. 89±4% suppression), but hepatic glucose output was fully and equally suppressed in both groups during the 40 and 1,200 mU/m^2 per min infusion. Finally, a significant inverse relationship was observed between the degree of glucose intolerance in the individual elderly subjects, as reflected by the 2-h serum glucose level during the oral glucose tolerance test, and the degree of peripheral insulin resistance as assessed by the glucose disposal rate during the 40 mU/m^2 per min insulin infusion ($r = 0.59$, $P < 0.01$).

We conclude that carbohydrate intolerance develops as part of the aging process. This carbohydrate intolerance appears to be the consequence of peripheral insulin resistance caused by a postreceptor defect in target tissue insulin action, which causes both a decrease in the maximal rate of peripheral glucose disposal and a rightward shift in the insulin action dose-response curve. In elderly subjects, the severity of the abnormality in carbohydrate tolerance is directly correlated to the degree of peripheral insulin resistance.

INTRODUCTION

The decrease in glucose tolerance that occurs with aging has been well documented (1, 2). Many investigators have demonstrated modest increases in plasma glucose levels after an oral glucose challenge (1, 2), and in his recent review of the literature, Davidson (1) concluded that the 2-h plasma glucose level during the oral glucose tolerance test appears to increase by a mean of 5.3 mg/dl per decade. Changes in fasting plasma glucose are more modest with an average increase of ~1 mg/dl per decade. Despite the fact that the decreased glucose tolerance has been widely described, relatively little is known about its mechanisms.

Most studies have demonstrated normal or increased insulin secretion as a function of aging following an oral or intravenous glucose load (3–7). However, some studies have demonstrated a delayed rise in insulin

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levels in the elderly during oral glucose tolerance testing and other investigators have shown a decrease in the early phase of insulin secretion following intravenous glucose tolerance tests (8–10). Because of the generally increased or normal insulin levels associated with elevated glucose levels during glucose tolerance tests, insulin resistance has been invoked as a mechanism responsible for the glucose intolerance of aging. Indeed, evidence in favor of insulin resistance has been presented by some investigators. Approximately 40 yr ago, Himsworth and Kerr (11) demonstrated a decrease in insulin sensitivity with aging using an oral glucose tolerance test with a simultaneous intravenous insulin injection (11). Although these conclusions were later substantiated by Silverstone et al. (12) and DeFronzo (13), the findings have not been universal. Kalant et al. (14) were unable to find any difference in insulin's ability to promote glucose uptake by muscle in elderly subjects. Similarly, Kimmerling et al. (15), using the steady-state plasma glucose response during the continuous infusion of standardized doses of insulin, glucose, epinephrine, and propranolol, as a measure of insulin resistance, found normal in vivo insulin action in a group of elderly subjects. Andres and Tobin (16) were also not able to demonstrate a decrease in insulin sensitivity as a function of aging. Therefore, we conducted the current study in an attempt to answer two questions: (*a*) does insulin resistance exist in elderly subjects? and, (*b*) if so, what are the underlying mechanisms?

METHODS

Materials. Porcine monocomponent insulin was generously supplied by Dr. Ronald Chance of the Eli Lilly & Co. (Indianapolis, IN); A^{14} monoiodinated insulin was supplied by Dr. Bruce Frank of the Eli Lilly & Co. [3-^{3}H]glucose was purchased from New England Nuclear (Boston, MA); bovine serum albumin (fraction V) was obtained from Armour Pharmaceutical Co. (Chicago, IL), collagenase was purchased from Worthington Biochemical Corp. (Freehold, NJ), guinea pig antiinsulin antibody was kindly supplied by Dr. Edward Arquilla (Irvine, CA).

Subjects. The study group consisted of 44 nonobese subjects. They were divided into two groups; an elderly group over the age of 60, and a nonelderly group under the age of 60. None of the subjects had impaired glucose tolerance as defined by the criteria of the National Diabetes Data Group (17). All subjects were healthy, ambulatory, and leading active lives. The clinical and metabolic characteristics of the subjects are summarized in Table I. Relative body weights were calculated as previously described (18). Lean body mass (LBM)[1] was calculated according to a modification of the formula of Moore et al. (19). This parameter was not significantly different for the women in the two groups, but the elderly men had a 9.6% decrease in LBM compared with the men in the nonelderly group ($P < 0.01$). Body mass index (BMI) was determined for each subject as the weight in kilograms per square centimeter $\times 10^{-3}$. After obtaining informed consent, all subjects were admitted to the University of Colorado Clinical Research Center but remained active to approximate their prehospital exercise level. All subjects were chemically euthyroid and had no stigmata of renal, hepatic, or cardiac dysfunction. With the exception of three subjects in the elderly group who were taking hydrochlorothiazide for hypertension, none of the other subjects was ingesting agents known to affect carbohydrate or insulin metabolism. The three subjects receiving diuretics had normal serum potassium levels, thereby ameliorating the effect of the drug on carbohydrate and insulin metabolism (20). Consistent with this formulation, no significant differences were noted between the data from these three subjects compared with the rest of the elderly group.

Diet. All subjects were placed on a weight-maintenance (30 Kcal/kg per d) liquid formula diet, with three divided feedings containing one-fifth, two-fifths and two-fifths of the total daily calories given at 0800, 1200, and 1700 h, respectively. The diet contained 45% carbohydrate, 40% fat, and 15% protein. All subjects were maintained on this diet for at least 48 h before studies were performed.

Oral glucose tolerance test. Oral glucose tolerance tests were performed by giving subjects 75 g glucose after an overnight fast. Serum was obtained at 0, 30, 60, 120, and 180 min for measurement of glucose and insulin levels.

Euglycemic glucose clamp studies. In vivo insulin action was assessed using a modification of the euglycemic glucose clamp technique as previously described (21–25) with the overall glucose disposal rate being measured isotopically for each 20-min interval of the study. After the initiation of the insulin infusion, steady-state rates of glucose disposal (defined as the first of three consecutive 20-min intervals with <5% change) were attained within 80–160 min, with the mean time for steady state being 100 min. The glucose disposal rates for the subsequent 60 min after achieving steady state were used as the data point for the individual study. Therefore, all studies were carried out for at least 140 min with some studies extending to a maximum of 220 min. The mean length of a study was 180 min. Urinary glucose loss was not a problem since these measurements were made under euglycemic conditions. The goal glucose was 85 mg/dl. The coefficient of variation of glucose during the period of steady-state was ±3%. The glucose clamp studies were done at three different insulin infusion rates; 15, 40, and 1,200 mU/m^2 per min. 11 subjects in both the nonelderly and elderly groups were studied at the insulin infusion rate of 15 mU/m^2 per min; 22 nonelderly subjects and 13 elderly subjects had glucose clamp studies performed at an insulin infusion rate of 40 mU/m^2 per min; and 10 nonelderly subjects and 13 elderly subjects had studies performed at an insulin infusion rate of 1,200 mU/m^2 per min. Each study was performed on a separate day. Overall a total of 80 studies were performed in these 44 subjects.

Hepatic glucose output. R_a, the rate of glucose appearance, and R_d, the rate of overall glucose disappearance, were quantified in both the basal state and during each of the glucose clamp studies by the infusion of [3-^{3}H]glucose in a primed continuous manner (25–27). With this technique, 25 μCi of the tracer is injected as a bolus, followed by a continuous infusion at the rate of 0.25 μCi/min. Blood samples are obtained at 20-min intervals beginning 60 min after the bolus injection for the determination of both the concentration and specific activity of serum glucose. R_a and R_d are

[1] *Abbreviations used in this paper:* BMI, body mass index(ces); LBM, lean body mass; R_a, rate of glucose appearance; R_d, rate of glucose disappearance.

then calculated using the Steele equations (28) in their modified derivative form (25, 26), since the tracer exhibits nonsteady-state kinetics under these conditions. In the basal state, R_a approximates hepatic glucose output since the liver is the predominant source of glucose during this time period. Unpublished data from our laboratory indicates that this approach gives analogous values for hepatic glucose output during the basal state as does the more traditional approach where tracer is infused until a steady state is achieved and steady-state assumptions used. During the glucose clamp studies, nonsteady-state conditions exist and R_a as calculated using the Steele equations represents the sum of residual hepatic glucose output and the rate of exogenous glucose infused.

Insulin binding studies. Insulin binding to isolated adipocytes was studied using cells obtained from an open biopsy of the adipose tissue on the lower abdominal wall. Details concerning the measurement and calculation of adipocyte insulin binding have been published previously (29, 30). Insulin binding to circulating monocytes was also studied using techniques previously described (29, 31).

Analytical methods. Blood for serum glucose determinations was drawn and serum immediately separated with a Beckman microfuge (Beckman Instruments, Inc., Fullerton, CA).

Blood for the determination of serum insulin levels and serum glucose specific activity was collected in untreated tubes and allowed to clot. The specimens were then spun, and the serum removed and stored at −20°C until the determinations were made. Serum insulin levels were measured by a double-antibody radioimmunoassay according to the method of Desbuquois and Aurbach (32).

Data analysis. All calculations were performed on a programmable calculator (model 67, Hewlett Packard Co., Palo Alto, CA). Data presented, unless otherwise stated, represent the mean±SE. Statistical analysis was done with Student's *t* test for paired data and unpaired data as indicated.

RESULTS

Oral glucose tolerance tests

As shown in Fig. 1, the mean fasting serum glucose level was not significantly different in the elderly group compared with the nonelderly subjects (88±2 vs. 84±2 mg/dl, respectively). 11 of the elderly had normal glucose levels throughout the test; the remaining six elderly subjects, while not meeting the established criteria for impaired glucose tolerance, exhibited nondiagnostic glucose tolerance tests according to the criteria of the National Diabetes Data Group (17). Consequently, when the glucose values for the elderly subjects were meaned, the values for the entire group were significantly greater than the respective values for the nonelderly subjects at 1, 2, and 3 h during the test (Fig. 1). When the data were analyzed by age category within the entire group, the 2-h postglucose value in the 19 subjects aged 20–39 was 108±6 mg/dl, 117±10 mg/dl in the 8 subjects aged 40–59 (NS), and 145±11 in the 17 subjects older than 60 ($P < 0.005$).

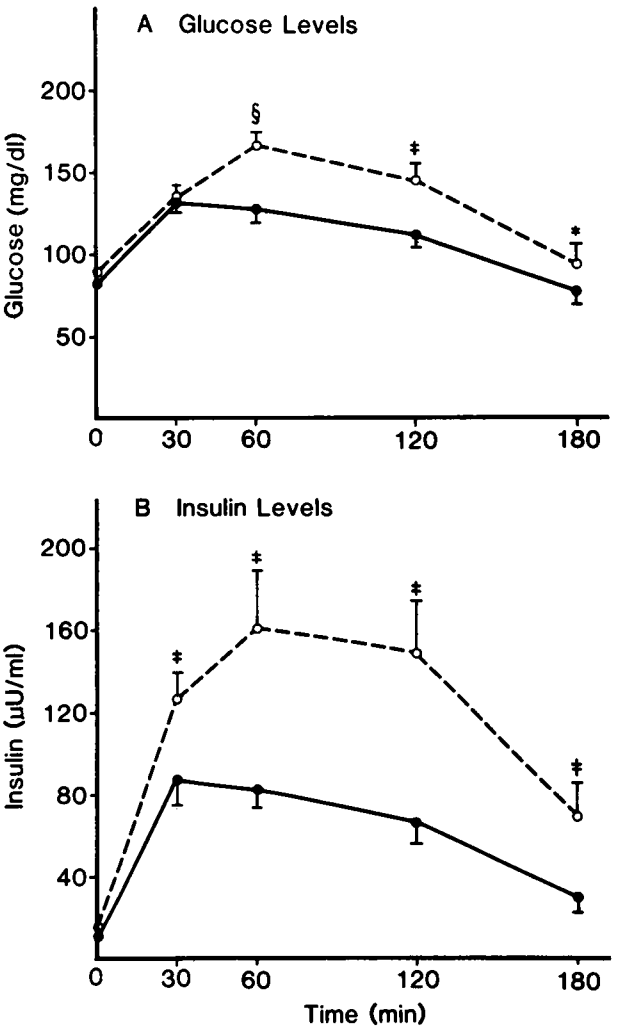

FIGURE 1 (A) Glucose levels during the oral glucose tolerance tests in nonelderly (●) and elderly (○) subjects. (B) Insulin levels during the oral glucose tolerance tests in nonelderly (●) and elderly (○) subjects. Results are plotted as mean±SEM.
* $P < 0.01$.
‡ $P < 0.005$.
§ $P < 0.001$.

In contrast to the fasting serum glucose levels, the fasting serum insulin levels were increased in the elderly compared with the nonelderly (13±1 vs. 9±1 μU/ml, $P < 0.005$). Following ingestion of the glucose load, the 1-, 2-, and 3-h mean serum insulin levels, like the corresponding glucose levels, were significantly higher in the elderly subjects ($P < 0.005$, Fig. 1). The presence of hyperglycemia in the elderly in the face of elevated insulin levels suggests an insulin-resistant state and this possibility was explored using the euglycemic glucose clamp technique.

Measurement of in vivo insulin sensitivity

To assess in vivo insulin sensitivity, euglycemic glucose clamp studies were performed using an insulin infusion rate of 40 mU/m^2 per min. The mean overall glucose disposal rate was decreased by 39% in the elderly group compared with the nonelderly (151±17 vs. 247±12 mg/m^2 per min, respectively, $P < 0.001$). The corresponding steady-state serum insulin levels attained during the studies were similar in both groups (110±5 μU/ml in the nonelderly and 102±8 μU/ml in the elderly). Therefore, the elderly group was clearly resistant to insulin's action to promote glucose uptake.

Because aging is a gradual phenomenon, it would seem reasonable to postulate that an inverse relationship should exist between overall glucose disposal rate and age. This relationship is shown in Fig. 2 A, and it can be seen that a statistically significant negative correlation exists between the glucose disposal rates obtained during the 40 mU/m^2 per min insulin infusion and the age of the subjects ($r = -0.53$, $P < 0.01$). However, this association is misleading if taken at face value. Thus, when the data are analyzed by dividing the entire group into three age categories [young (20–39), middle (40–59), and elderly (60+)] it can be seen (Fig. 2 B) that the insulin resistance is only observed in the elderly group (60+ yr) and that no decrease in in vivo insulin action to promote glucose uptake exists in the middle age group.

Mechanism of insulin resistance

Dose-response relationship. To assess the mechanisms responsible for the insulin resistance seen in the elderly, glucose clamp studies were also performed at insulin infusion rates of 15 and 1,200 mU/m^2 per min (Fig. 3). The 15 mU/m^2 per min insulin infusion yielded steady-state serum insulin concentrations of 33±2 μU/ml in the nonelderly and 61±5 μU/ml in the elderly. Despite the higher insulin concentrations achieved, the glucose disposal rate was markedly decreased (39%) in the elderly (93±7 mg/m^2 per min) compared with the nonelderly (152±11 mg/m^2 per min) ($P < 0.001$). At the insulin infusion rate of 1,200 mU/m^2 per min, steady-state serum insulin levels were similar in the two groups (11,316±890 μU/ml vs. 11,083±1,079 μU/ml) and previous studies have shown that this insulin level results in maximally stimulated glucose disposal rates. Maximal insulin stimulated glucose disposal rates were decreased by 25% in the elderly (328±24 mg/m^2 per min) compared with the nonelderly (439±21 mg/m^2 per min) ($P < 0.005$). Thus, the elderly group displayed a decrease in maximal insulin responsiveness, indicative of a postreceptor defect in insulin action (25). In addition to this decrease in insulin responsiveness, there was also a decrease in insulin sensitivity in the elderly group as manifested by the fact that there was a 39% decrease in insulin's action to promote glucose uptake in the elderly subjects at the low dose (15 mU/m^2 per min) insulin infusion, a 39% decrease during the 40 mU/m^2 per min insulin infusion, and only a 25% decrease during the high dose (1,200 mU/m^2 per min) insulin in-

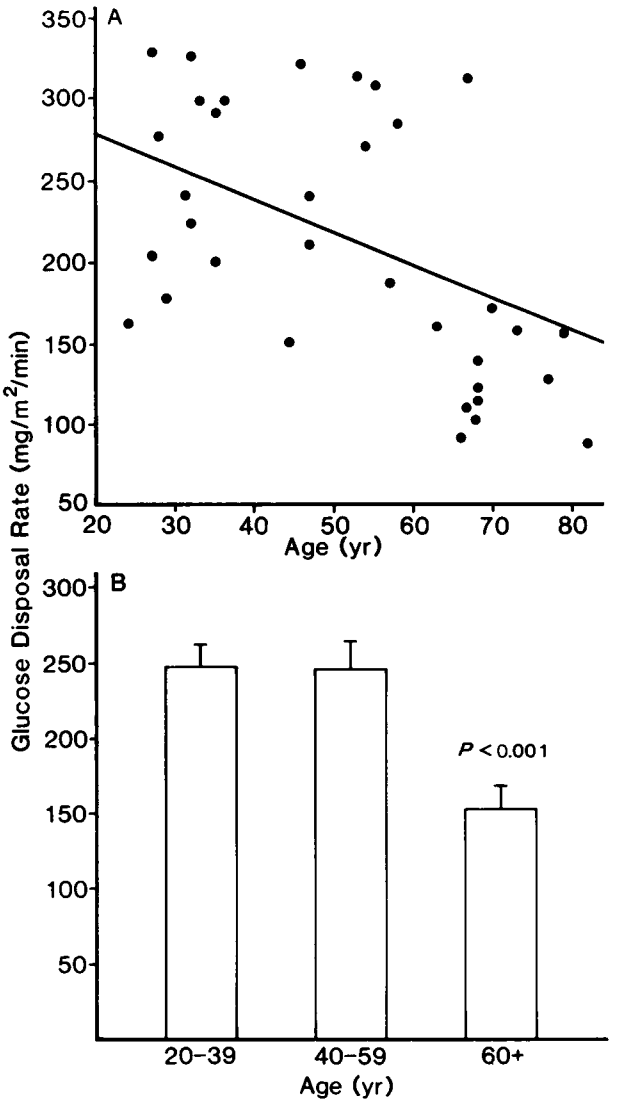

FIGURE 2 (A) Relationship between the glucose disposal rate at an insulin infusion rate of 40 mU/m^2 per min and age of the subjects showing a significant inverse correlation ($r = -0.53$, $P < 0.01$). (B) Mean glucose disposal rates at an insulin infusion rate of 40 mU/m^2 per min with the subjects divided into three age groups. The number of subjects in each age group was 13 for the 20–39 yr; 8 for the 40–59 yr; and 13 for the 60+ yr. Values represent the mean±SEM.

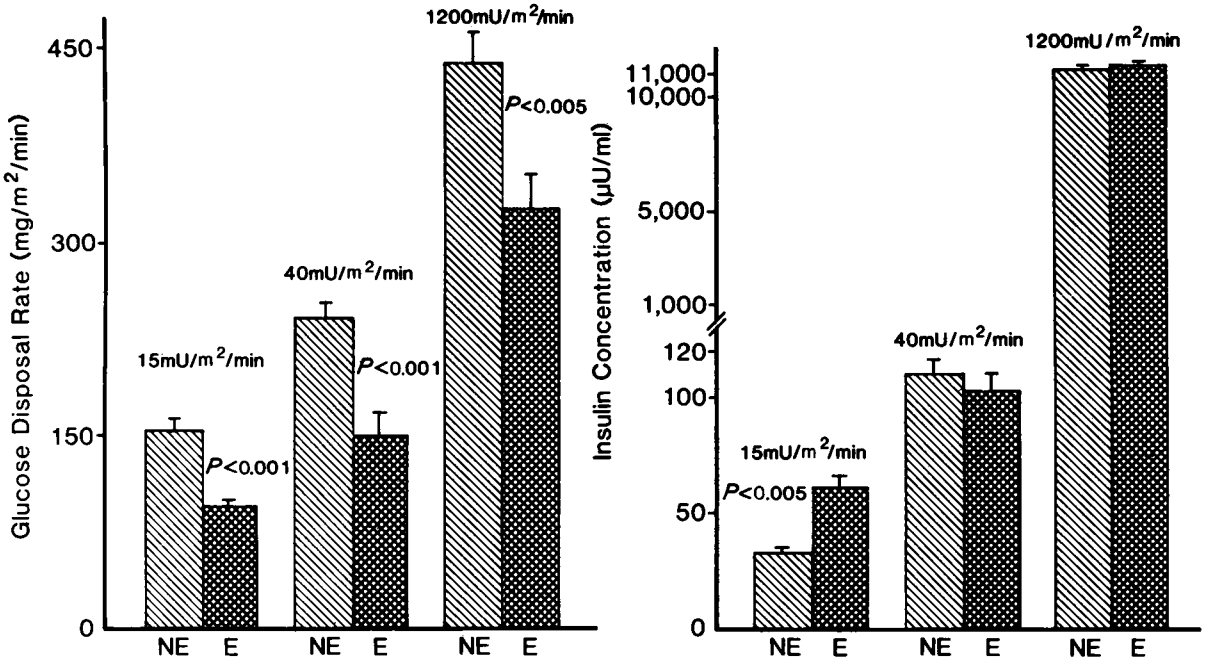

FIGURE 3 (A) Mean dose-response relationship for nonelderly (NE) and elderly (E) subjects. Results are displayed as mean±SEM of glucose disposal rates for insulin infusion rates of 15, 40, and 1,200 mU/m² per min. (B) Steady-state serum insulin concentrations during the three insulin infusion studies.

fusion. Thus, the decrease in insulin action was greater at the lower insulin concentrations than the higher one, indicative of a rightward shift in the dose-response curve. This point is made stronger in view of the fact that the steady-state insulin level during the low dose infusion was greater (61±5 μU/ml) in the elderly compared with the nonelderly (33±2 μU/ml). This concept is further illustrated in Fig. 4 where the data are plotted as a percentage of the maximal insulin effect. For each group, the maximal glucose disposal rate was taken as 100%, and the glucose disposal rate at each submaximal insulin level is plotted as a percentage of this value. For purposes of this analysis, 70% of the absolute basal glucose disposal rate is initially subtracted from all values because this represents non-insulin-mediated glucose uptake (21). Fig. 4. shows an approximate twofold rightward shift in the dose-response curve of the elderly group compared with the nonelderly group.

It should be noted that there were no significant differences in our data between the men and women in each of the nonelderly or elderly groups. Thus, glucose disposal rates in the elderly men were 89±8 vs. 103±15 mg/m² per min in the elderly women at an insulin infusion rate of 15 mU/m² per min (NS). Similarly, there were no significant differences at insulin infusion rates of 40 mU/m² per min (elderly men: 148±19 mg/m² per min vs. elderly women: 158±20 mg/m² per min) and 1,200 mU/m² per min (elderly men 322±33 vs. elderly women 341±17 mg/m² per min).

Since the elderly men had a 9.6% decrease in LBM (Table I) compared with the nonelderly, it could be

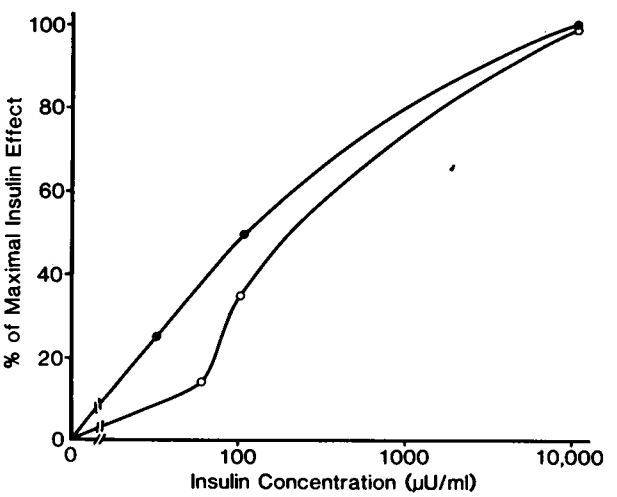

FIGURE 4 Mean dose-response curves for the nonelderly (●) and elderly (○) subjects, plotted as the percentage of maximal insulin effect.

TABLE I
Clinical and Metabolic Features

	Nonelderly	Elderly
Subjects (*n*)	27	17
Sex (M)	7	11
(F)	20	6
Mean age (±SE) (*yr*)	37±2	69±1
Age range (*yr*)	23–58	60–82
Mean relative weight	0.94±0.02	0.93±0.02
LBM (*kg*) M	58.2±1.5	52.6±1.3 ($P < 0.01$)
F	39.3±1.0	37.8±1.4 (NS)
BMI (kg/cm^2) × 10^{-3}	2.29±0.05	2.40±0.05 (NS)

argued that this accounted for the observed decrease in glucose disposal rate when the data are normalized to unit surface area (m^2), as in Figs. 2 and 3. However, when glucose disposal rates were expressed per unit of LBM, the elderly group still evidenced insulin resistance with decreases in overall glucose disposal rates of 37, 38, and 26% at insulin infusion rates of 15, 40, and 1,200 mU/m^2 per min, respectively, when compared with the nonelderly subjects (Fig. 5).

As can be seen in Fig. 5, although the mean glucose disposal rates were decreased in the elderly group at the three insulin infusion rates, some of the elderly subjects had peripheral glucose disposal rates similar to the nonelderly group. Analysis of the individual data revealed that at one end of this spectrum were those 11 elderly subjects with normal glucose tolerance tests (closed circles, Fig. 5), and at the other end were the 6 elderly subjects with nondiagnostic glucose tolerance tests (open circles, Fig. 5). As seen in Fig. 6 A, although all the elderly subjects were insulin resistant, the mag-

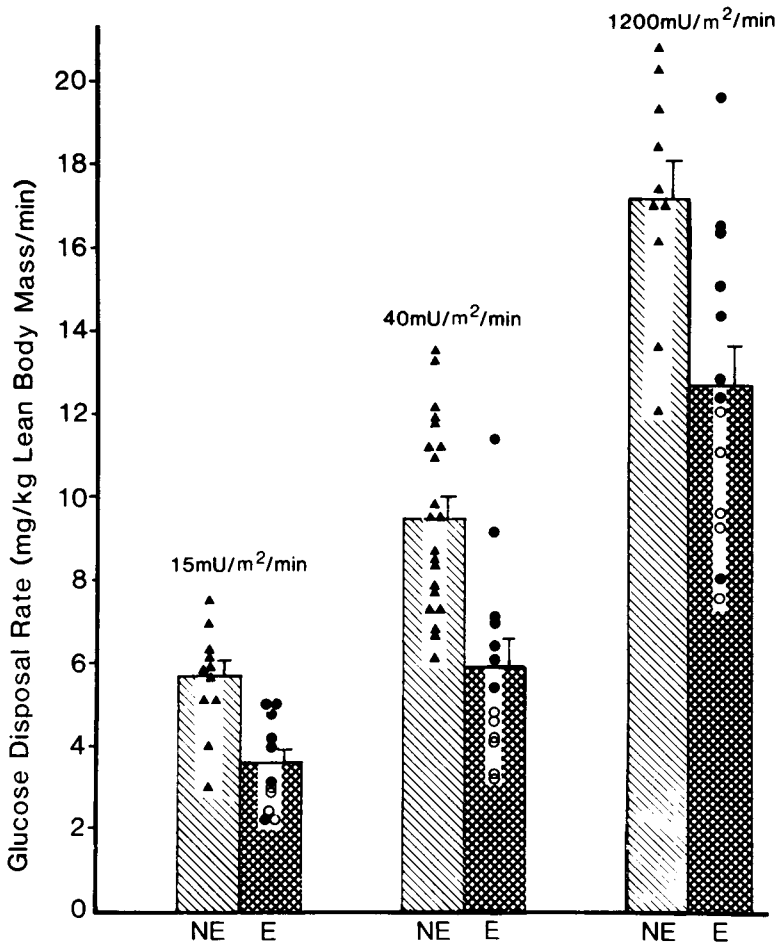

FIGURE 5 Dose-response relationship of glucose disposal rates for insulin infusion rates of 15, 40, and 1,200 mU/m^2 per min expressed per unit of LBM. Data are displayed as mean±SEM for nonelderly (NE), and elderly (E). Individual subjects are depicted as NE (▲), elderly with normal glucose tolerance tests (●), and elderly with nondiagnostic glucose tolerance tests (○).

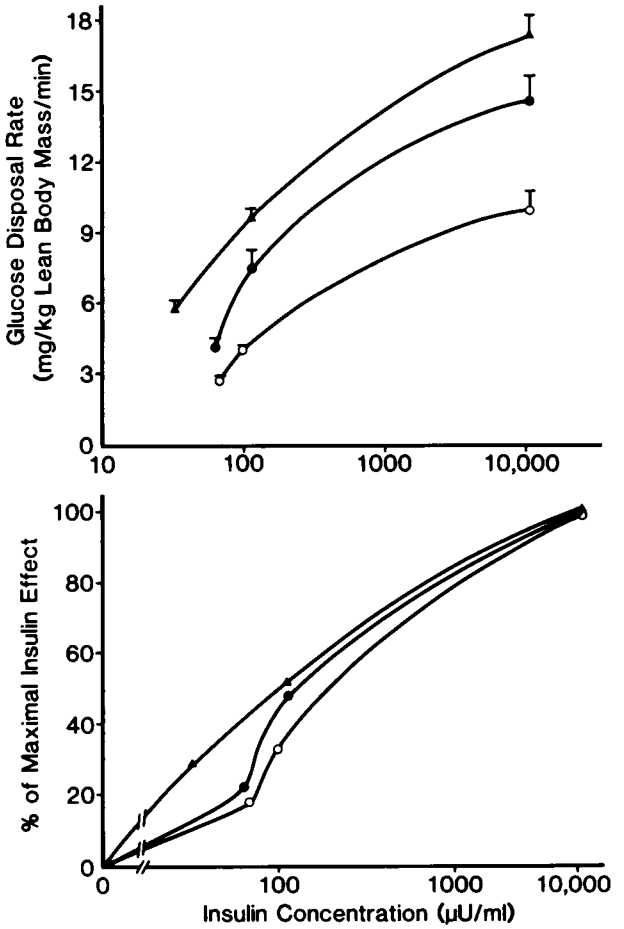

FIGURE 6 (A) Mean dose-response curves for nonelderly (▲), elderly with normal glucose tolerance tests (●), and elderly with nondiagnostic glucose tolerance tests (○). (B) Mean dose-response curves for the three groups plotted as the percentage of maximal insulin effect.

nitude of this defect was much greater in those subjects with nondiagnostic glucose tolerance tests. This group had decreases in overall glucose disposal rates of 53, 58, and 42%, compared with the nonelderly group, while the elderly group with normal glucose tolerance tests had decreases of 28, 21, and 16% compared with the nonelderly group at insulin infusion rates of 15, 40, and 1,200 mU/m^2 per min, respectively. Additional evidence for this continuum can be seen in Fig. 6 B where the data are shown as a percentage of the maximal insulin effect. The elderly group with nondiagnostic glucose tolerance tests had a greater rightward shift in the dose-response curve compared with the elderly with normal glucose tolerance tests. Fig. 7 shows the relationship between a measurement of the elderly subjects' glucose intolerance, namely the 2-h glucose levels during the oral glucose tolerance test, and a measurement of in vivo insulin action, namely the glucose disposal rate during the 40 mU/m^2 per min glucose clamp study. The correlation between these two variables is quite good ($r = -0.59$, $P < 0.05$) indicating that as insulin resistance increases with aging a corresponding decline in glucose tolerance occurs.

Adipocyte and monocyte binding. Fig. 8 (left panel) presents the competition curves for insulin binding to isolated adipocytes from the elderly and nonelderly groups. It is evident that these curves are essentially identical for the two groups suggesting that insulin binding to receptors is unaffected by aging. It should be noted however, that these data are expressed on the basis of cell number and some reports have suggested that receptor density per unit of cell surface area is a more appropriate method of expressing insulin binding data (29). This is especially pertinent, since the adipocytes from the elderly group were 16% larger in volume than those from the nonelderly group (393±33 pl vs. 332±48 pl). The difference in surface area, however, was only 10% (2.56×10^4 μ^2/cell in the elderly group vs. 2.29×10^4 μ^2/cell in the nonelderly group). When the insulin binding data were normalized to cell surface area, the binding curves were still comparable (Fig. 8, right panel) and no significant differences were noted at any insulin concentration. To further explore this issue, insulin binding to monocytes was measured and these results are shown in Fig. 9. Again, no difference in the ability of cells from the elderly vs. nonelderly group to bind insulin was observed, and in this cell system, aging had no effect on cell size.

Hepatic glucose output. Basal hepatic glucose output was similar in both groups (79±3 vs. 78±4 mg/m^2 per min in nonelderly and elderly groups, respectively; Fig. 10). However, insulin's ability to suppress hepatic glucose output was decreased in the elderly compared with the nonelderly during the low dose insulin infusion rate of 15 mU/m^2 per min. Thus, hepatic glucose output was suppressed by 89±4% in the nonelderly subjects compared with only 77±5% in the elderly ($P < 0.05$). This difference in suppressibility of hepatic glucose production is even more impressive in light of the finding that the low dose insulin infusion yielded higher steady-state serum insulin levels in the elderly compared with the nonelderly (61±5 μU/ml vs. 33±2 μU/ml). At an insulin infusion rate of 40 mU/m^2 per min, hepatic glucose output was equally suppressed in both groups (95±2 vs. 93±3%). And, during the 1,200 mU/m^2 per min insulin infusion rate, hepatic glucose output was similarly maximally suppressed (98 vs. 100%). The decreased suppressibility at low concentrations of insulin with normal maximal suppressibility at higher insulin levels indicates a rightward

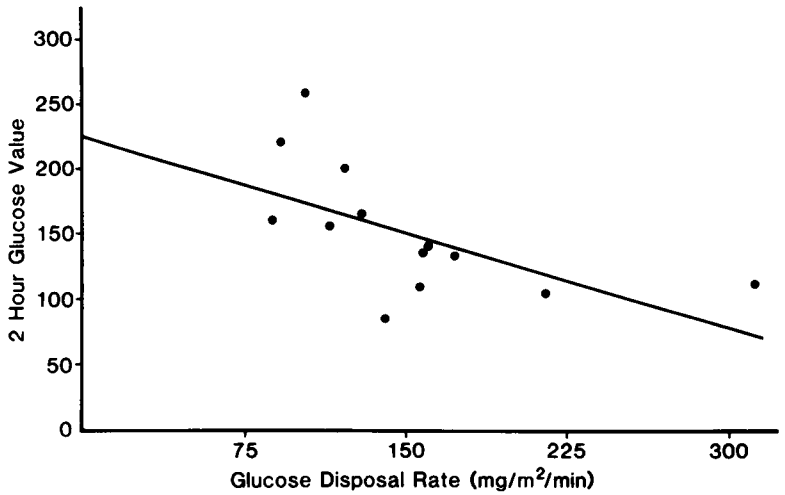

FIGURE 7 Relationship between the 2-h glucose value during the oral glucose tolerance test and the glucose disposal rate during the 40 mU/m² per min insulin infusion in the elderly group. A significant inverse correlation was observed, $r = -0.59$, $P < 0.05$.

shift in the dose-response curve for insulin's ability to suppress hepatic glucose production in elderly subjects.

DISCUSSION

We have studied the effects of aging on carbohydrate metabolism across a continuum of nonelderly and elderly healthy, nonobese subjects. The elderly population overall had decreased glucose tolerance following an oral glucose load and had increased insulin secretion in response to the glucose challenge. This confirms previous studies, which demonstrated declining glucose tolerance with age (1, 2). The elevated insulin levels in the face of increased glucose levels

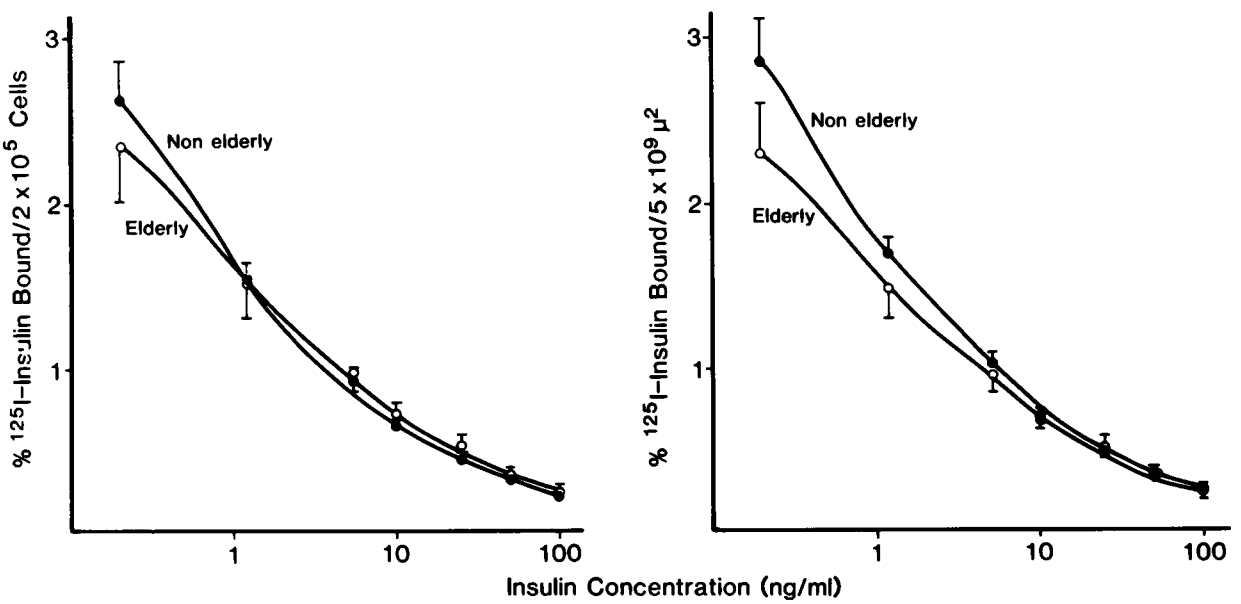

FIGURE 8 Left panel: Insulin binding by isolated adipocytes from nonelderly subjects (●) and elderly subjects (○). All data are corrected for nonspecific binding and represent the mean±SEM of the percentage ^{125}I-insulin specifically bound per 2×10^5 cells. Right panel: Insulin binding by isolated adipocytes expressed as the mean±SEM of the percentage ^{125}I-insulin specifically bound per $5 \times 10^9 \mu^2$.

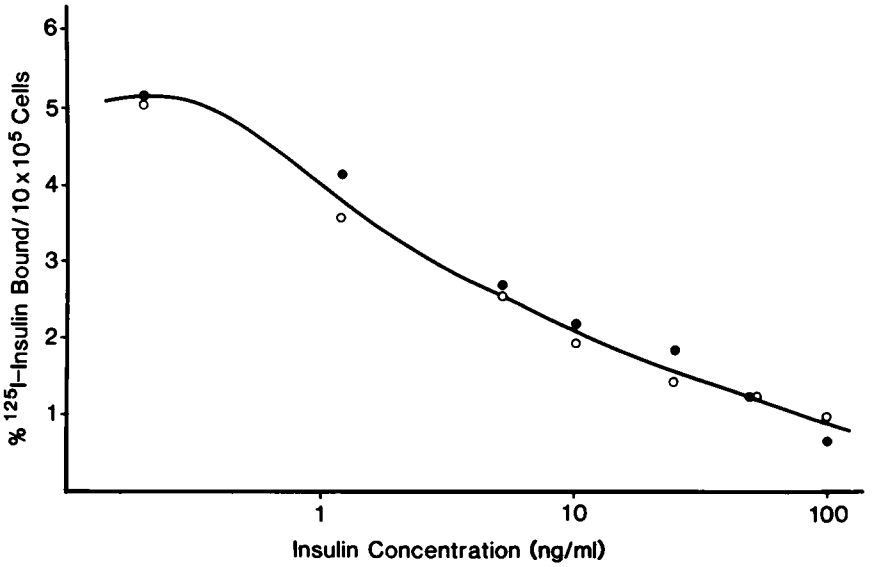

FIGURE 9 Insulin binding by circulating monocytes from 10 nonelderly (●) and 10 elderly (○) subjects. All data represent the mean±SEM of the percentage ^{125}I-insulin specifically bound per 10×10^5 cells.

suggest an insulin resistant state. This was confirmed by utilizing the euglycemic glucose clamp technique that demonstrated resistance to insulin's action to promote glucose uptake in elderly subjects. During physiological hyperinsulinemia (insulin concentrations $\cong$ 100 μU/ml) the elderly group had a 39% decrease in overall glucose disposal rate compared with the nonelderly group. The mechanisms underlying the insulin resistance of aging have, to date, been unclear. The results of the glucose clamp studies performed at insulin concentrations of 15, 40, and 1,200 mU/m^2 per min suggest that aging is associated with a postreceptor

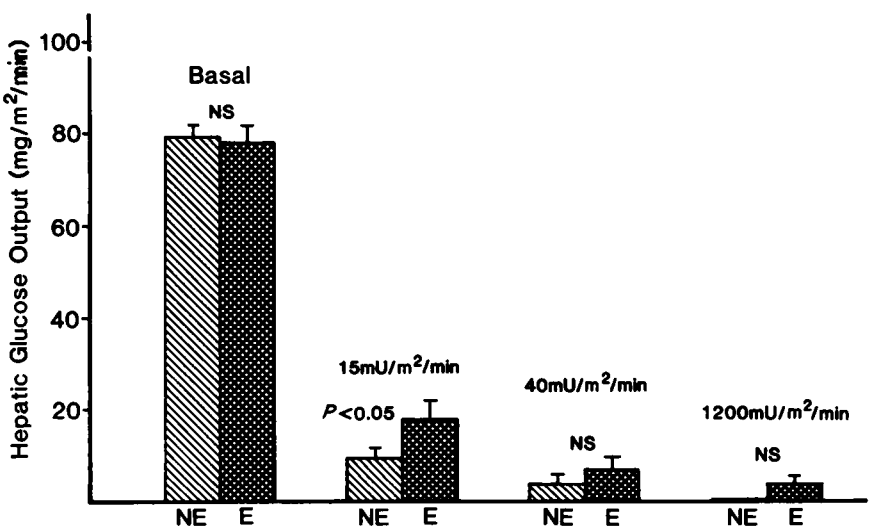

FIGURE 10 Mean dose-response relationship for insulin-mediated suppression of hepatic glucose output in nonelderly (NE) and elderly (E) subjects. The initial bars farthest left represent glucose output in the basal state as determined by the primed continuous infusion of [3-^{3}H]glucose. The bar for the nonelderly (NE) group at the far right indicates 100% suppression of hepatic glucose output. Data are represented as mean±SEM.

defect in insulin action. Thus, the elderly group was not able to attain glucose disposal rates comparable to the nonelderly group at the highest insulin infusion rate of 1,200 mU/m^2 per min, and this rate yielded insulin concentrations of $\cong$ 11,000 μU/ml, which in previous studies (21, 25) have been shown to generate the maximal possible insulin effect. An inability to achieve a normal maximal insulin effect is termed decreased insulin responsiveness and implies a postreceptor defect in insulin action. Insulin binding to isolated adipocytes and circulating monocytes was unchanged with advancing age, which further confirms the presence of a postreceptor defect in insulin action.

In addition to the decrease in maximal insulin responsiveness, an apparent rightward shift in the insulin dose-response curve exists (Fig. 4). The elderly population demonstrated a 39% decrease in the peripheral glucose disposal rate during the low dose (15 mU/m^2 per min) insulin infusion, a 39% decrease during the 40 mU/m^2 per min infusion, and a 25% decrease during the 1,200 mU/m^2 per min infusion. This smaller percentage decrease in insulin's ability to stimulate glucose uptake at the highest insulin level compared with the lower ones indicates a rightward shift in the dose-response curve for insulin action. This rightward shift is even more pronounced if one takes into account the fact that the steady-state serum insulin levels during the lowest insulin infusion rate (15 mU/m^2 per min) were almost twice as high in the elderly (61±5 μU/ml) compared with the nonelderly (33±2 μU/ml). Thus, if the steady-state serum insulin levels had been comparable, a much greater difference in the rates of glucose disposal would have been observed between the two groups. The reason the elderly displayed higher steady-state serum insulin levels at the low insulin infusion rate is not clear, but this could be due either to impaired metabolic clearance of the infused insulin or incomplete suppression of endogenous insulin secretion, or both.

A rightward shift in the dose-response curve is termed a decrease in insulin sensitivity and is usually due to a decrease in insulin binding to its target tissue (21, 33). However, since insulin binding to isolated adipocytes and circulating monocytes was normal in the elderly group, these current results indicate that the rightward shift in the dose-response curve in the elderly subjects represents a decrease in insulin sensitivity due to a postreceptor (or postbinding) defect in insulin action and is not due to a decrease in insulin binding. Similarly, insulin's ability to suppress hepatic glucose output was decreased in the elderly at low insulin concentrations but was normal at high insulin levels, which maximally and completely suppress hepatic glucose output. Again, this difference in hepatic insulin action at the low insulin infusion rate is more striking in view of the fact that the elderly achieved twofold higher insulin levels during these studies. Therefore, a decrease in insulin sensitivity (rightward shifted dose-response curve) in the elderly is seen at the level of hepatic glucose production, and this is comparable to the decrease in sensitivity for insulin's effects to stimulate glucose disposal. If one assumes that adipocyte and monocyte insulin receptors are reflective of the status of hepatocyte insulin receptors, then this decrease in insulin sensitivity is not due to decreased insulin binding, but rather to a postreceptor defect in insulin action. A possible mechanism to explain a rightward shift in the dose-response curve due to a postreceptor abnormality would be a coupling defect distal to insulin binding. If such a defect caused this step to become rate limiting in insulin action in elderly subjects, then even at low levels of receptor occupancy, this defect would be expressed as a rightward shift and be of a postreceptor (or postbinding) nature.

The present results also indicate that there is a spectrum, or continuum, of insulin resistance in the elderly. Thus, there was a strong negative correlation between the degree of insulin resistance and the magnitude of the glucose intolerance in the individual elderly subjects. Based on analysis of the individual data, we believe that in nonobese, nondiabetic elderly subjects, the degree of insulin resistance ranges between those subjects with normal and those with nondiagnostic glucose tolerance tests. Some elderly individuals have completely normal glucose tolerance tests with mildly elevated insulin levels, indicating insulin resistance. These subjects have maximal insulin-stimulated glucose disposal rates that are near normal, but have markedly decreased glucose disposal rates at lower insulin levels indicating a rightward shift in the dose-response curve (decreased insulin sensitivity) (Fig. 6 A). Insulin binding to adipocytes and monocytes is normal in these individuals, and therefore, the insulin resistance manifested in this group indicates a type of postreceptor defect with near normal maximal insulin action but with decreased insulin effects to stimulate glucose disposal or inhibit hepatic glucose production at lower insulin concentrations. 11 of the 17 elderly subjects fell into this category. The mean maximal glucose disposal rate in these 11 subjects was 368±31 mg/m^2 per min. When this value was corrected for the 9.6% decrease in LBM exhibited by men in this group compared with the nonelderly men, a small but significant (16%) difference existed between nonelderly and elderly (17.2±0.9 vs. 14.4±1.2 mg/kg LBM/min). The insulin resistance was more pronounced during the 15 and 40 mU/m^2 per min insulin infusion rates in these 11 elderly subjects with normal glucose tolerance, since the glucose disposal rates were de-

creased by 28 and 21%, respectively, in the elderly compared with the nonelderly (Fig. 6 A). At the other end of the spectrum lie those elderly individuals who have nondiagnostic glucose tolerance tests according to National Diabetes Data Group criteria (17). These subjects also have greater elevations of serum insulin levels, normal insulin binding, and marked insulin resistance manifested as both a decrease in insulin action at submaximal and maximal levels. 6 of the 17 elderly subjects fell into this group and they had a 53, 58, and 42% decrease in glucose disposal rates compared with the nonelderly group at insulin infusion rates of 15, 40, and 1,200 mU/m^2 per min, respectively (Fig. 6 A). This analysis is not presented to indicate the existence of two distinct groups in the elderly population but to point out that there appears to be a continuum of metabolic abnormalities ranging from mild to more severe insulin resistance and that the basic lesion is a postreceptor defect in insulin action that manifests itself predominantly as decreased insulin sensitivity when insulin resistance is mild, and decreased insulin sensitivity plus decreased responsiveness when the insulin resistance is more severe. This spectrum of insulin resistance resembles to some degree that seen in other insulin resistance states of obesity and noninsulin-dependent diabetes mellitus. However, the mechanisms underlying the insulin resistance appear to differ. In obesity there is a spectrum of insulin resistance ranging from mild to more severe and associated with a rightward shift in the dose-response curve in the former and a rightward shift plus decreased responsiveness in the latter (25). In obese subjects there is a decrease in insulin binding, well correlated to the magnitude of the rightward shift (25). Similarly, previous studies indicate that subjects with impaired glucose tolerance have decreased insulin sensitivity due to a decrease in insulin binding, whereas patients with noninsulin-dependent diabetes mellitus have both decreased insulin sensitivity and responsiveness (21). This contrasts with our current study where elderly subjects with normal glucose tolerance have a rightward shift in the dose-response curve, but have normal insulin binding to both adipocytes and monocytes leading us to conclude that the rightward shift is due to a postbinding defect. One can speculate then that the difference in mechanisms responsible for the rightward shift in aging compared with obesity and impaired glucose tolerance resides in the fact that the former case is a physiologic change due to the aging process and the latter are pathophysiologic states.

Previous studies examining tissue sensitivity to insulin as a function of aging have yielded conflicting results. Utilizing a combined infusion of insulin, glucose, epinephrine, and propranolol, Kimmerling et al. (15) were unable to demonstrate a decrease in insulin sensitivity in elderly subjects. However, all of the subjects in their study had normal glucose tolerance tests, and it seems likely that their group represented one end of the spectrum discussed above. Using the forearm perfusion technique, Kalant et al. (14) did not find a decrease in insulin-stimulated muscle glucose uptake with advancing age. However, only 1 of the 31 subjects they studied was over the age of 60. Thus, it is not surprising that no age-related decline in insulin action was seen, and their results are in agreement with our data showing no insulin resistance in the middle age range. DeFronzo (13) has used the euglycemic glucose clamp technique and reported results similar to ours in a group of elderly subjects, although he found that the decrease in insulin sensitivity could also be demonstrated in the 30–50-yr-age group as well. Robert et al. (34) also concluded that reduced glucose tolerance in the elderly is associated with a decrease in the uptake of glucose by peripheral tissues. Finally, Andres and Tobin (16) have reported normal glucose disposal rates as measured with the euglycemic clamp method in elderly subjects.

Interpretation of the decline in glucose tolerance and presence of insulin resistance in aging has been made difficult because of associated changes that occur in diet, activity level, and degree of adiposity. Diets low in carbohydrate will impair glucose tolerance (35). If the elderly subjects had a substantial decrease in their carbohydrate intake, this could conceivably have resulted in abnormal glucose tolerance tests on the basis of diet alone. However, Seltzer (35) has analyzed the effect of diet on the percentage of abnormal oral glucose tolerance tests in an older population. Although proportionately more glucose tolerance tests were normal as the carbohydrate in the diet increased, a larger percentage still remained abnormal, and when matched for carbohydrate intake, the elderly subjects still had significantly higher glucose values than a younger population, even though the test results were within the normal range.

Another dietary factor that may play a role in the glucose intolerance of aging is the level of organic chromium in the diet. Marginal dietary intake of chromium over years in man can lead to a depletion of the body's chromium content (36–38), and many elderly people are so affected. There have been several studies indicating that a deficiency of chromium may play a role in the insulin resistance and glucose intolerance of aging, and supplementation of chromium in the diet of elderly individuals has been shown to improve glucose tolerance (39–41). It seems possible, therefore, that chromium deficiency may play a role in the insulin resistance of aging.

Physical activity has also been shown to affect insulin sensitivity. Increases in maximal aerobic power

are correlated to an increase in insulin's ability to promote glucose uptake (42, 43). Since elderly individuals are generally less active than younger individuals, it is possible that the glucose intolerance of aging is at least partly due to decreased physical fitness. However, this explanation seems unlikely in the current study group since all of our elderly subjects were ambulatory, active, and in several instances involved in regular strenuous physical training programs. We do not, however, discount the possibility that the degree of physical training may play some role in the spectrum of the insulin resistance of aging.

It could be argued that some of the effect of aging on carbohydrate metabolism is not due to the aging process itself, but to the increase in percent adiposity that occurs with aging. It is known that both body fat (44, 45) and fat cell size increase with age, and, therefore, separating the effects of age and obesity could be a potential problem. For these reasons we analyzed the data from the glucose clamp studies by normalizing the results to both body surface area (Fig. 3) and to LBM (Figs. 5 and 6). The elderly subjects in our study all had relative body weights and BMI comparable to the nonelderly group and LBM differed by only 9.6% in the elderly men, while no difference in LBM was observed in women. Therefore, although a small component of the decrease in in vivo insulin action may be accounted for on the basis of increased adiposity, and decreased LBM, it would not explain the large 30–40% differences in glucose uptake between nonelderly and elderly. This is emphasized in Figs. 5 and 6, which show that even when the glucose disposal rates were corrected for the small differences in LBM, marked insulin resistance was still readily evident in the elderly group. Similarly, despite comparable relative weights, adipocytes size was greater in the elderly compared with the nonelderly (393 pl/cell vs. 332 pl/cell, respectively). However, when insulin binding to adipocytes was expressed per unit of cell surface area, no difference in insulin binding was noted between the nonelderly and elderly groups. This observation is reinforced by the data in Fig. 9 which show no effect of aging on insulin binding to isolated circulating monocytes (a cell type whose size does not change with age).

In conclusion, we have evaluated the mechanisms underlying the glucose intolerance of aging. Aging is associated with a spectrum of insulin resistance. On one end are those elderly individuals who display no abnormalities in glucose tolerance testing, are mildly hyperinsulinemic, and have insulin resistance manifested as a mild postreceptor defect in insulin action with a rightward shift in the insulin action dose-response curve with near normal maximal insulin action. On the other end of the spectrum are those individuals who are glucose intolerant, hyperinsulinemic, and whose insulin resistance consists of a more severe postreceptor defect expressed as a more marked rightward shift in the dose-response curve and a decrease in maximal insulin action.

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